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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
09/262,126	03/03/1999	BRIAN S. MILLER	GC396-2	8961

5100 7590 06/29/2005

GENENCOR INTERNATIONAL, INC.
ATTENTION: LEGAL DEPARTMENT
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EXAMINER	
RAO, MANJUNATH N	

ART UNIT	PAPER NUMBER
1652	

DATE MAILED: 06/29/2005

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	09/262,126	MILLER ET AL.	
	Examiner	Art Unit	
	Manjunath N. Rao, Ph.D.	1652	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *17 May 2005*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *5-10,12,14,15,27-40 and 52-66* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *5-10,12,14,15,27-40 and 52-66* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: _____.

DETAILED ACTION

The finality of the previous Office action has been withdrawn. In the previous Office action, claims 8, 30 and 54 were inadvertently indicated as objected and were not included in the rejection. Therefore, this Office action is being issued in order make it of record that claims 8, 30 and 54 are also rejected. Any inconvenience caused is deeply regretted.

Claims 5-10, 12, 14-15, 27-40, 52-66 are currently pending in this application.

Applicants' amendments and arguments filed on 5-17-05, have been fully considered and are deemed to be persuasive to overcome the rejections previously applied. Specifically Examiner has withdrawn the rejections under 35 U.S.C. 112, 2nd paragraph is withdrawn in view of claim amendments.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

Claims 5-10, 14-15, 27-40, 52-61, 63-66 are rejected under 35 U.S.C. 103(a) as being unpatentable over Deweer et al. (US 6,074, 854 filed 12-23-97, issued 6-13-2000) and McPherson et al. (Biochemical Soc. Trans., 1988, vol. 16(5) :723-724) or Albertson (Biochim. Biophys. Acta, Vol. 1354:35-39, 1997).

This rejection is based on printed publications and a patent. Claims 5-10, 14-15, 27-40, 52-61, 63-66 in this instant application are drawn to a modified pullulanase from *B.deramificans* T89.117D with an amino acid sequence of SEQ ID NO:2, wherein the modification is a deletion of about 98, 100, 102, 200 amino acids from the amino terminus, wherein the modified pullulanase is produced by culturing a host cell comprising a nucleic acid which is at least 90% identical to SEQ ID NO:1 encoding a truncated pullulanase wherein the host cell is *B.licheniformis* in which certain proteases are inactivated or eliminated. The claims are also drawn to compositions comprising the above modified pullulanase and compositions further comprising additional enzymes such as glucoamylase isolated from *Aspergillus* strains and wherein the modified pullulanase is 60 or 80% of the composition and wherein the composition is in the solid or liquid form.

Deweer et al. teach a pullulanase obtained from a Gram positive bacteria such as *B.deramificans* T89.117D produced by a method of culturing a host cell such as *B.licheniformis* in which certain protease genes have been inactivated. The reference also teaches the method of making the recombinant enzyme by obtaining the host cell transformed with a polynucleotide having more than 90% identity to SEQ ID NO:1 (see sequence alignment sent in the previous office action). The reference teaches the compositions either in the solid form or liquid form comprising pullulanase wherein it is of the order of 60% of the total enzyme concentration. The reference also teaches compositions comprising additional enzymes such as glucoamylase isolated from *Aspergillus* strains (see claims in the reference). However, the reference does not teach modification of pullulanase by way of deletion of about 100, 200 or 300 N-terminal amino acids.

McPherson et al. teach that pullulanases are significantly large enzymes when compared to other polysaccharide hydrolases and that this large size reduces the efficiency with which it can function by restricting access to internal alpha 1,6 bonds within highly branched substrates. The reference teaches that proteolytic digestion and computer-based sequence analyses are being used in the art to define a functional "core" pullulanase. The reference provides sources for such computer based homology searches. As an example the reference provides a schematic illustration of the relative position of the 5 conserved "amylase" regions within a selection of hydrolases in comparison to the large *K.pneumoniae* pullulanase. The reference teaches that the long N-terminal region lacks any polysaccharide binding or catalyzing sites. McPherson et al. teach the modification of deleting nearly 170 amino acid residues from the amino terminal end which leads to approximately 30% higher activity than that of the native enzyme.

Albertson et al. also teach the modification of a pullulanase (from *C.saccharolyticus*), wherein nearly 381 nucleotides from the 5' region of the cDNA encoding a pullulanase was deleted resulting in a N-terminal truncated pullulanase. The reference also teaches that the deleted amino acid sequence is not essential for either activity or thermostability.

While both McPherson et al. and Albertson et al. do not teach a pullulanase isolated from a *Bacillus*, it appears that experiments involving truncation of N-terminal amino acids in pullulanase enzymes was well known in the art. These experiments appear to have been performed to determine the nature and the location of secretion signal, activity, catalytic site, transport across membrane and secretion into liquid medium.

It would have been obvious to one skilled in the art at the time the invention was made to combine the teachings of Deweer et al. with that of McPherson et al. and Albertson et al. to

compare the large pullulanase provided by Deweer et al. with other *Bacillus* pullulanase just as taught by McPherson et al., followed by a method to make a modified pullulanase in which any number of amino acids up to at least a maximum of 381 amino acids from the N-terminal amino acids have been deleted. This is because Deweer et al. teach a pullulanase isolated from a Bacillus, *B.deramificans*, which is a very large size enzyme with more than 900 amino acids. McPherson et al. teach a method of increasing the efficiency of large size pullulanase by determining and deleting non-essential amino acids in the N-terminal region. Albertson et al. and McPherson et al. demonstrate that deletion of up to at least 170 and 381 amino acids in such large size pullulanases does not affect the activity of the enzyme negatively but on the other hand increases the efficiency of the enzyme by nearly 30%. It would also be obvious for one skilled in the art to eliminate or inactivate protease genes in the expression hosts, such as Carlsberg protease or endo Glu C protease as Deweer et al. teach such inactivation of proteases such that the heterologous protein is not digested by the endogenous proteases.

Based on the above teachings, one of ordinary skill in the art would be motivated to delete N-terminal amino acids just as McPherson et al. by comparing and determining that N-terminal regions of large pullulanase do not have any conserved sequences for either activity or binding to polysaccharide and cleavage of such non-essential sequences results in higher efficiency of the enzyme. Those of ordinary skill in the art would also be motivated by Albertson et al. teaching in which up to 381 N-terminal amino acids have been deleted. One of ordinary skill in the art would have a reasonable expectation of success since Deweer et al. provide the nucleic acid encoding the pullulanase from *B.deramificans* in a host cell such as *B.*

licheniformis in which protease genes have been inactivated and also provide the compositions comprising up to 60% of pullulanase in order to perform the modification.

Therefore the claimed invention would have been *prima facie* obvious to one of ordinary skill in the art.

In response to the rejection of the above claims, applicants have traversed the above rejection and argue at length that the invention is not rendered obvious by the references. In summary applicants appear to be arguing that the enzymes in the reference and that claimed have divergent sources and different amino acid lengths and have different conserved sequences that it would not have been obvious for those skilled in the art to arrive at the conclusion of truncating the instant enzyme which has its source in Bacillus sp. Applicants point out that the enzyme in the reference is from a Gram negative bacteria (as in McPherson et al.) as opposed to the Gram positive bacteria in the instant claims. Next applicants argue that in the absence of extensive homology with the reference proteins it would not have been possible for those skilled in the art to arrive at the instant invention. With reference to the enzyme of Albertson et al. also, applicants seem to make an issue out of this that the reference enzyme shares only 35% amino acid sequence identity and that the number of amino acids truncated is different from what the Examiner has alluded to. Applicants conclude that they who have found larger deletion mutants than that shown in the prior art are capable of retaining enzymatic activity and that they have defined the maximum extent of such deletion which the enzyme can tolerate. Examiner respectfully disagrees that the above arguments are persuasive to overcome the rejection. Examiner agrees that the references teach diverse species and that the amino acid sequence homology is as low as 35% between the reference enzyme and the instant claimed enzyme.

However, therein lies the obviousness as well. The reference indeed teaches that irrespective of the source, pullulanase enzyme tends to be a large enzyme with unnecessary amino acid stretches which can be removed. The diverse source of the enzyme indeed leads those skilled in the art to be more definitive that similar truncation in a bacillus would work and contrary to applicant's argument, it would not direct those skilled in the art away from drawing such a conclusion.

Examiner vehemently disagrees with applicants' conclusion that an "obvious to try" standard has been used in determining the patentability of the present invention. According to applicant's own assertion, an obviousness rejection is inappropriate wherein the prior art [gives] either no indication of which parameters [are] critical or no direction as to which of many possible choices is likely to be successful. In the instant case, prior art gives ample direction and provides the critical parameters and reaction to proceed in truncating the pullulanase. However, it is the applicant who takes support in ambiguous characteristics (the so called conserved sequence) or parameters in determining that claims are not obvious.

Therefore, for all the above reasons, Examiner continues to maintain the above rejection of claims under 35 U.S.C. 103(a) as being *prima facie* obvious.

Claim 12 is rejected under 35 U.S.C. 103(a) as being unpatentable over Deweer et al. (US 6,074, 854 filed 12-23-97, issued 6-13-2000). This rejection is based upon the public availability of a patent publication. Claim 12 of the instant application is drawn to a modified pullulanase isolated from *B.deramificans*, wherein the modification is an addition of at least has at least one amino acid added to the amino terminus of a mature pullulanase amino acid sequence, wherein the added amino acid is alanine. Deweer et al. teach the modification of an

identical mature pullulanase by at least one amino acid, i.e., addition, substitution, deletion of at least one amino acid. However, the reference does not specifically teach that the added amino acid need to be alanine.

However, with the above pullulanase in hand followed by the teaching of modifying it by at least one amino acid, it would have been obvious to those skilled in the art to modify the enzyme of Deweer et al. by adding one amino acid anywhere in the sequence including the N-terminal and assay such modified enzymes of having the pullulanase activity. Since there are only twenty amino acids that can be used for modification, it would be obvious to those skilled in the art to use all or any of the twenty amino acids including alanine and select one or more of the modified enzyme that continues to have the activity. One of ordinary skill in the art would be motivated to do so in order to make a pullulanase that is simply different from that of an already patented enzyme in the art. One of ordinary skill in the art would have a reasonable expectation of success since there are only a limited number of amino acids that can be used for modification of an enzyme and Deweer et al. provide the mature pullulanase enzyme and also teach that a modification with at least one amino acid can be made along with techniques that can be used for making such modified enzyme.

Therefore, the above invention would have been *prima facie* obvious to those skilled in the art.

Applicants have not specifically addressed the above rejection. However, Examiner assumes that their arguments were indeed directed to the above rejection as well. In response Examiner draws their attention to the above arguments by the Examiner in support of the previous rejection.

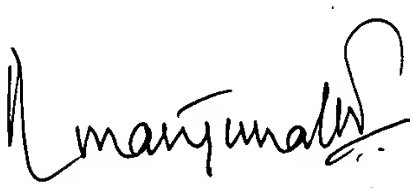
This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(f) or (g) prior art under 35 U.S.C. 103(a).

Conclusion

None of the claims are allowable.

Any inquiry concerning this communication or earlier communications from the Examiner should be directed to Manjunath N. Rao, Ph.D. whose telephone number is 571-272-0939. The Examiner can normally be reached on 7.00 a.m. to 3.30 p.m. If attempts to reach the examiner by telephone are unsuccessful, the Examiner's supervisor, Ponnathapura Achutamurthy can be reached on 571-272-0928. The fax phone numbers for the organization where this application or proceeding is assigned is 571-273-8300 for regular communications and for After Final communications.

Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the receptionist whose telephone number is 571-272-1600.



Manjunath N. Rao, Ph.D.
Primary Examiner
Art Unit 1652

June 15, 2005